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Does shell powder from Anomalocardia flexuosa enhance emergence and initial development in seedlings of *Crotalaria juncea* L.?[1]

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ABSTRACT - Although the use of alternative substrates in seedling production is still uncommon, the aim of this study was to evaluate the effect of different particle sizes and concentrations of shell powder from *Anomalocardia flexuosa* on emergence and initial biomass production in seedlings of *Crotalaria juncea*, varying the composition of the basic substrate components. The experimental design was of randomised blocks in a factorial scheme that included three particle sizes (3 mm, 2 mm and 250 µm), five concentrations (0%, 20%, 40%, 60% and 80%) of shell powder, and three basic substrate constituents (soil, vermiculite and organic compost), giving a total of 45 treatments, with four replications per treatment, in which each experimental plot consisted of 25 seedlings. The following variables were evaluated: seedling emergence, emergence speed index, plant height, number of leaves, collar diameter, dry matter accumulation, H/CD ratio (shoot height to collar diameter), SDW/RDW ratio (shoot dry weight to root dry weight) and H/SDW ratio (shoot height to shoot dry weight). The particle size of the shell powder and the basic constituents of the substrates had no significant effect on the variables under analysis. Regardless of particle size or concentration, the shell powder enhanced the emergence, shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) of the *C. juncea* seedlings.

Key words: Sustainability. Mollusc shells. Caatinga. Alternative substrate.

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INTRODUCTION

The substrate is one of the most important components of seedling production and is essential for retaining water, supplying nutrients and developing the root until the seedling is transplanted into the field (Macumbi *et al*., 2023). Organic materials and agro-industrial waste, such as coconut fibre and carbonised rice husks, can be used for this purpose. These materials improve the quality of the substrate, promoting seedling growth and an increase in biomass, by helping to retain moisture and aerate the substrate (Griebeler *et al*., 2023).

The composition of the substrate can vary as required, and consists of either a single material or a combination of various components (Salles *et al*., 2024). Due to the price and availability of the materials, the ideal formulation can result in high costs (Orta-Guzmán *et al*., 2021). Recent studies have suggested different by-products as alternatives for use as substrates.

Among these, sludge (Monteiro *et al*., 2019), sugarcane bagasse (Orta-Guzmán *et al*., 2021) and coconut fibre (Macumbi *et al*., 2023) can be used as alternatives for reducing costs and minimising the negative environmental impact resulting from the disposal of waste material. Shellfishing is a prominent source of waste in many parts of the world, as the shells are discarded and lead to the accumulation of large quantities of inert material.

The shells of *A. flexuosa* are mainly composed of calcium carbonate, which has various agricultural uses (Kang *et al*., 2017), with a better absorption rate and greater biological potential than inorganic minerals (Leão *et al*., 2020). Shell powder in the form of nanoparticles can be used for treating seeds, germination and seedling development (Anand *et al*., 2021; Zhang *et al*., 2023). Calcium is an essential macronutrient that plays a vital role in maintaining cell walls, structuring membranes, and regulating plant growth (Anand *et al*., 2021; Thor, 2019).

However, it is necessary to evaluate the material when used as part of a substrate, especially in terms of germination and initial plant growth. The aim of this study was to evaluate the effect of different particle sizes and concentrations of *Anomalocardia flexuosa* shell powder, and of varying the composition of the basic constituents of the substrates, on emergence and initial biomass production in seedlings of *C. juncea*.

MATERIAL AND METHODS

The shells were collected manually in the district of Arez, Rio Grande do Norte (6º09’46” S and 35º07’21” W).

Immediately after collection, the shells were packed in plastic bags and taken to the seed laboratory of the Specialised Agricultural Sciences Unit - EAJ/UFRN. The shells were removed from the bags, washed by hand in running water until no visible impurities remained, and dried for 72 hours in a circulation oven at 65 °C to completely remove any moisture. The shells were then crushed in a forage

Figure 1 - Waste from shells of *Anomalocardia flexuosa* discarded near the mangrove swamp in Arez, RN, after extracting the mollusc

Source: Research data, 2024

grinder (TRF 400), which produced the first particle size, 3 mm. Part of this material was ground in a knife mill (CIENLAB, CE-430) and then separated using a 2 mm sieve to obtain the second particle size. The material was further separated using a 250 µm sieve to produce even finer particles, which made up the third of the particle sizes: G1 – 3 mm, G2 – 2 mm and G3 – 250 µm. Before incorporating the shell powder into the base constituents, a quantitative analysis of the chemical composition of the shells was carried out by X-ray powder diffraction (Table 1).

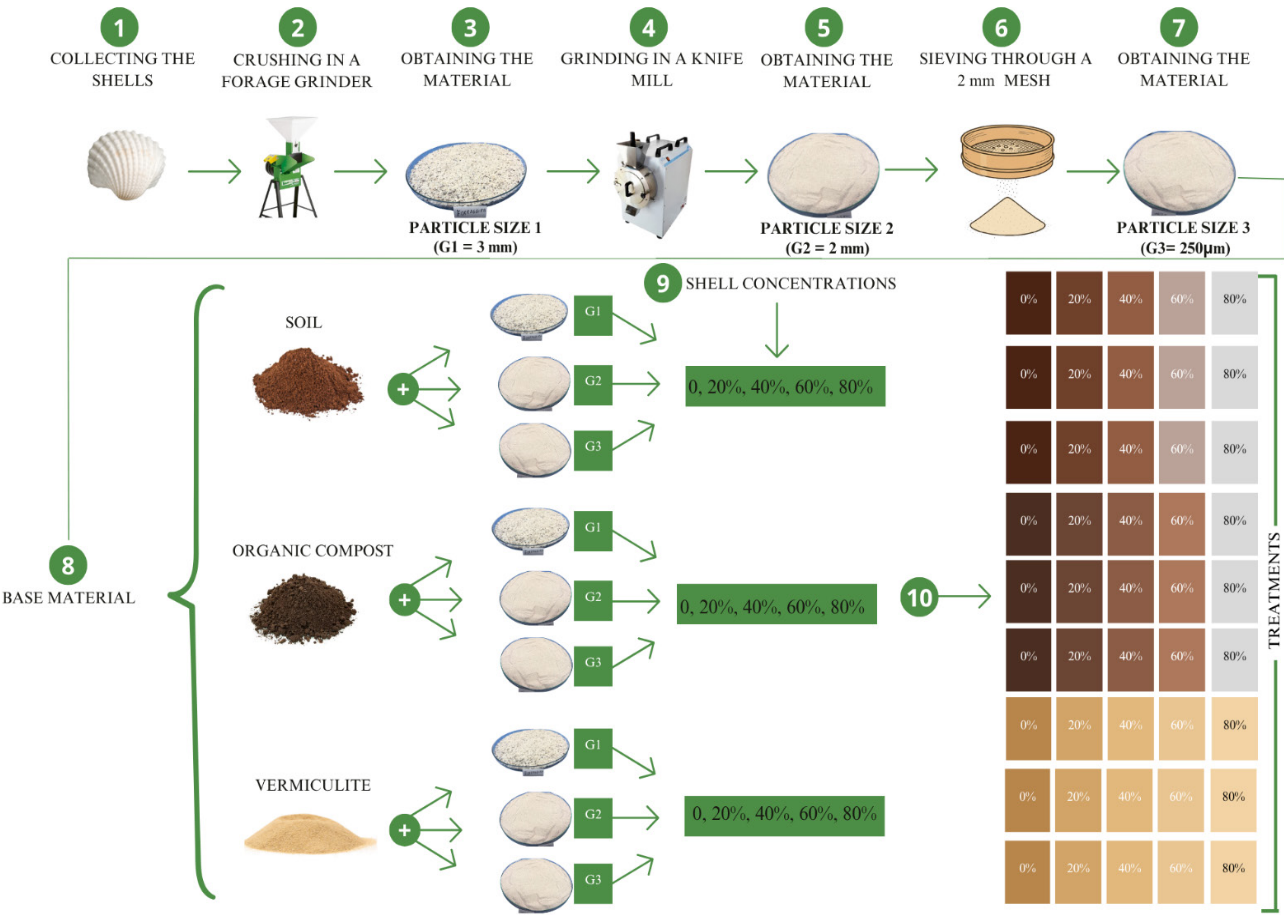
The basic constituent of the substrate for each mixture included either soil characterised as an Argisol with a sandy texture, vermiculite or organic compost. Three particle sizes and five concentrations of shell powder (0%, 20%, 40%, 60% and 80%) were used for each combination, with four replications of 25 seeds in each treatment. Sowing was carried out in polyethylene trays of 200 cells, using two seeds per cell (Figure 2).

Table 1 - Chemical composition of the powder from *Anomalocardia fllexuosa* shells determined by diffraction. The values represent the percent by weight of the oxides identified in the sample

Chemical composition	Weight (%)
CaO	96.03
SiO_2	1.41
SO_3	1.15
Fe_2O_3	0.57
SrO	0.44
K_2O	0.26
ZnO	0.11
Ag_2O	0.02

CaO = calcium oxide; SiO_2 = silicon dioxide; SO_3 = sulphur trioxide; Fe_2O_3 = iron oxide; SrO = strontium oxide; K_2O = potassium oxide; ZnO = zinc oxide; Ag_2O = silver oxide

Figure 2 - Diagram of the process for obtaining *Anomalocardia flexuosa* shell powder, showing the treatments with different particle sizes (G1 - 3 mm, G2 - 2 mm and G3 - 250 µm) in different concentrations (0%, 20%, 40%, 60% and 80%) added to different base materials (soil, vermiculite and organic compost)



Irrigation was carried out on alternate days to maintain the soil moisture close to field capacity, which was determined by saturating, draining and then weighing the substrate. The irrigation depth was adjusted throughout the experiment based on periodic weighing to maintain the ideal moisture level, as per Firmino (2014). The emerged seedlings presented two fully open and normal cotyledons. Counts were made daily for ten days (Brasil, 2013), after which emergence stabilised.

The emergence speed index was determined from a daily count of the emerged seedlings, and calculated as per Maguire (1962). A digital calliper was used to measure the diameter of the collar at ground level (mm); the number of leaves was determined by counting; shoot height was determined with the aid of a millimetre rule, measuring from the base of the collar to the apical bud of the plant.

The shoots and roots of the seedlings were sectioned with the aid of pruning shears, packed in Kraft paper bags and placed in a forced air circulation oven at 65 °C; the bags were then weighed on a precision analytical balance. The total dry weight was determined as the sum of the shoot and root dry weight. The H/CD ratio (shoot height to collar diameter), SDW/RDW ratio (shoot dry weight to root dry weight) and H/SDW ratio (shoot height to shoot dry weight) were determined by dividing the respective variables.

The experimental design was of randomised blocks in a 3 x 3 x 5 factorial scheme, with three particle sizes of shell powder, three basic substrate constituents and five shell powder concentrations, with four replications per treatment, in which each experimental plot consisted of 25 seedlings. Analysis of variance was applied, considering significance levels of 5% and 1% probability using the F-test. Whenever the differences were significant, the data were submitted to regression analysis using the R software, v. 3.5.1 (R Core Team, 2018).

RESULTS AND DISCUSSION

As shown in Table 2, only the concentration of *A. flexuosa* shell powder had a significant effect ($P \leq 0.01$) on emergence (E), emergence speed index (ESI), diameter (D) and number of leaves (NL).

The substrate must provide the right conditions, as it influences water retention capacity and nutrient absorption, and can therefore have an impact on germination and initial seedling development (Fang *et al*., 2022). The analysis of variance of seedling emergence revealed a gradual increase, showing a significant effect from the shell powder concentrations regardless of the base constituent of the substrate. The *C. juncea* seedlings had an percentage emergence of over 90% when sown in a substrate containing 80% shell powder (Figure 3).

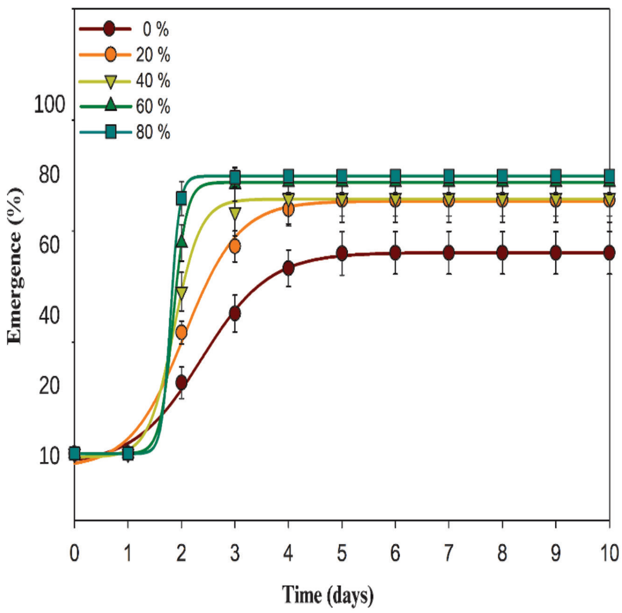
Similar results to those of this study were reported by Paim *et al*. (2022), who found that only the substrate produced significant results in all the variables under analysis. During seedling production, calcium stimulates faster initial growth, resulting in a decrease in the time required for production (Menegatti *et al*., 2017). Anand *et al*. (20210 reported a positive effect from shell powder on the seeds of *Vigna radiata*, showing that nanoparticles of $CaCO_3$ as a source of nutrients afforded a significant increase in germination and the germination speed index.

Table 2 - Analysis of variance for emergence (E), emergence speed index (ESI), diameter (D) and number of leaves (NL) in seedlings of *Crotalaria juncea* under different concentrations of *Anomalocardia flexuosa* shell powder, basic constituents and particle sizes

Source of variation	DF	Mean square			
		E	ESI	D	NL
Particle size (P)	2	38.8^{ns}	4.944^{ns}	0.003^{ns}	1.106^{ns}
Basic constituent (B)	2	147.8^{ns}	3.588^{ns}	0.024^{ns}	2.489^{ns}
Concentration (C)	4	4289.7**	169.526**	2.530**	259.592**
B x P	4	58.5^{ns}	1.433^{ns}	0.024^{ns}	0.781^{ns}
B x C	8	47.5^{ns}	0.977^{ns}	0.025^{ns}	1.162^{ns}
G x C	8	6.4^{ns}	1.222^{ns}	0.014^{ns}	0.425^{ns}
B x P x C	16	27.8^{ns}	1.462^{ns}	0.025^{ns}	1.308^{ns}
Residual	135	56.4	1.658	0.019	1.376
C.V. (%)		8.2	13.14	7.9	14.8

ns, not significant; *, **- significant at $p \leq 0.05$ and $p \leq 0.01$ by F-test, respectively

Figure 3 - Emergence percentage in seedlings of *Crotalaria juncea* in response to variations in particle size and concentration of *Anomalocardia flexuosa* shell powder

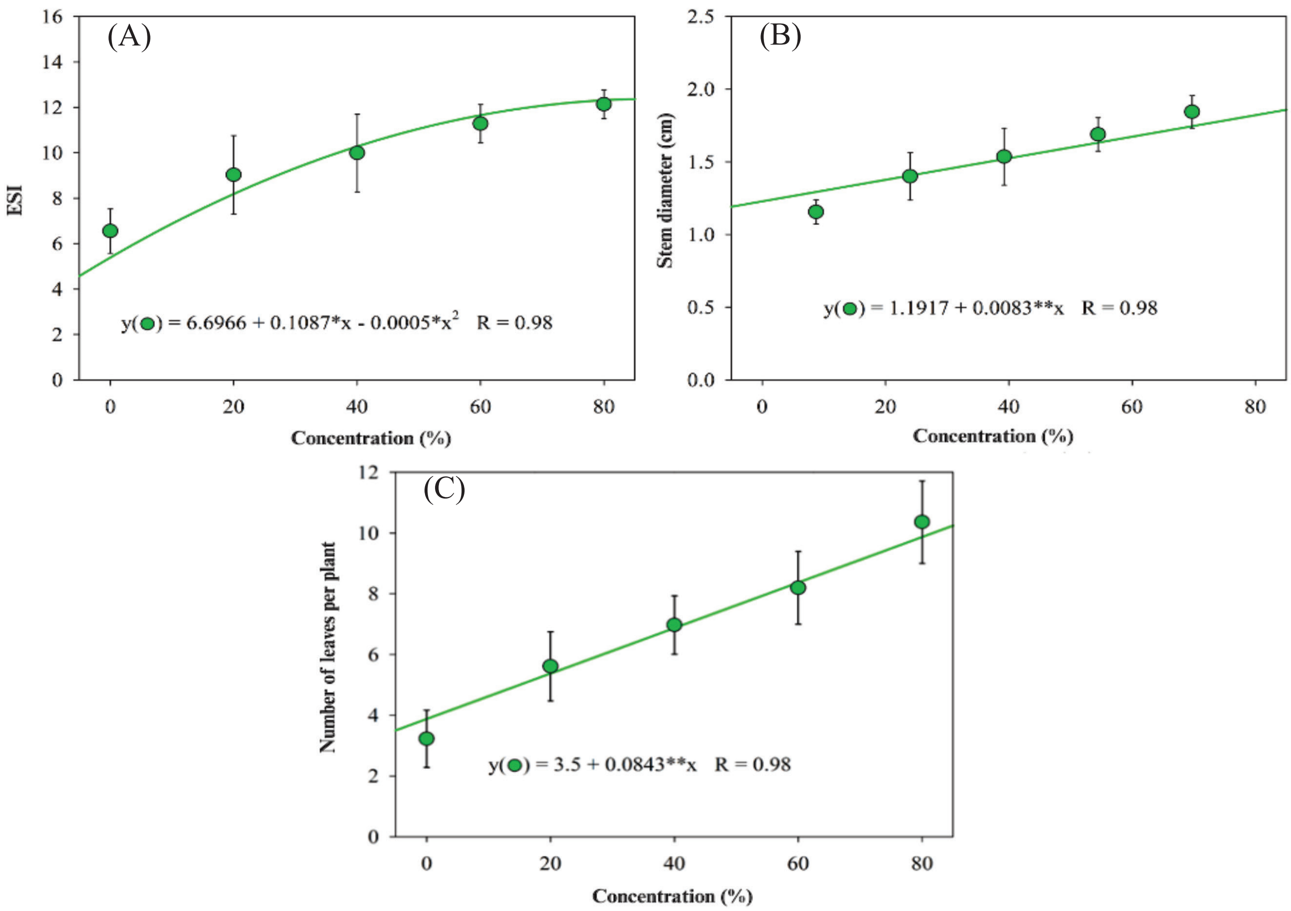


The results show that the emergence of the *C. juncea* seedlings and the speed of the process were both positively influenced by the increasing concentrations of shell powder in the substrate. The shell powder concentration had a quadratic effect on the emergence speed index, with a maximum point of 12.92 at a concentration of 80% and 6.69 for the control.

Linear behaviour was seen for the diameter and number of leaves of the *C. juncea* seedlings as the shell powder concentration increased. The stem diameter showed a gradual increase from 1.19 mm, when the concentration was 0%, to 1.35, 1.52, 1.68 and 1.85 mm when the concentration was 20%, 40%, 60% and 80%, respectively. Similar behaviour was seen for the number of leaves, with respective values of 3.5 and 10.24 at the minimum and maximum concentrations (Figure 4).

The emergence speed index can be an important metric in evaluating the quality of seeds and seedlings, and also serves as an indirect indicator of seed vigour. As such, the direct correlation between the tests of vigour and emergence in the field is important (Rego *et al*., 2023).

Figure 4 - Emergence speed index (A), stem diameter (B) and number of leaves (C) in seedlings of *Crotalaria juncea* under different concentrations of *Anomalocardia flexuosa* shell powder



Calcium absorption occurs mainly via the soil, where elements in the soil-plant system are directly related to the presence of calcium in soluble form, replenishing the soil solution as levels are reduced through loss or absorption by plants. The positive effect on emergence can therefore be attributed to the strong link between calcium and root growth (Thor, 2019).

The collar diameter is an important variable in seedling production, as it is an extremely reliable indicator of plant quality. A thicker collar affords the seedlings support and stability, with a positive effect on vigorous growth (Marques *et al*., 2018). In relation to the number of leaves, it is important to point out that using a substrate with an adequate supply of nutrients in the nursery environment plays a decisive role in the rapid increase in leaf area of developing seedlings (Smiderle *et al*., 2016). Similar results were reported by Paim *et al*. (2022) who found that, for *Lupinus bracteolaris*, the stem diameter showed an upward quadratic trend that was proportional to the amount of fertiliser in the substrate.

The concentration of the shell powder was decisive for the responses seen in the variables under analysis, since all the variables showed a significant interaction ($P \leq 0.01$): shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW). The different concentrations had a different effect on the plant characteristics. On the other hand, no significant interactions were found between the material and the particle size of the shell powder (Table 3).

Seedling length is one of the main tests for evaluating seedling performance and, consequently, the effect of the seed substrate (Guedes *et al*., 2015). Analysis of the average length of the normal seedlings shows that individuals that have the highest values are the most vigorous (Nakagawa, 1999). This results from the greater translocation of reserves from the storage tissues to support growth of the embryonic axis. Accordingly, under all the experimental conditions, the seedlings developed as the concentration of the shell powder increased, regardless of particle size.

Increasing linear behaviour was seen for shoot height, shoot dry weight and root dry weight in the *C. juncea* seedlings for the different concentrations; while for root length, the data followed an upward quadratic trend, proportional to the concentration of powder in the substrate (Figure 5).

At the highest concentration (80%), there was an increase of 17.69 and 5.15 compared to the control (0% concentration), demonstrating the positive effect on seedling growth. There was no significant variation in root length between the treatments (Figure 6).

Similar behaviour to seedling length was seen for shoot and root dry weight, where the shell powder afforded the greatest dry weight, of 2.19 and 0.86 g, respectively (Figure 7).

The application of shell powder favoured the accumulation of biomass, resulting in a significant increase in shoot and root dry weight during initial growth. Similar results were reported by Cunha *et al*. (2023) for *Leucaena leucocephala*, where calcium had a positive effect on development. There was no significant interaction between the particle size of the shell powder and the base material of the substrate for either the shoot height to collar diameter ratio (H/SD), shoot height to shoot dry weight ratio (H/SDW) or

Table 3 - Summary of the analysis of variance for shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) in seedlings of *Crotalaria juncea*

Source of variation	DF	Mean square			
		SL	RL	SDW	RDW
Particle size (P)	2	14.30^{ns}	4.751^{ns}	0.251^{ns}	0.063^{ns}
Basic constituent B)	2	10.46^{ns}	12.709^{ns}	0.160^{ns}	0.014^{ns}
Concentration (C)	4	895.12**	79.073**	15.884**	1.914**
B x P	4	7.01^{ns}	9.584^{ns}	0.044^{ns}	0.036^{ns}
B x C	8	4.38^{ns}	11.752^{ns}	0.103^{ns}	0.024^{ns}
G x C	8	8.07^{ns}	9.176^{ns}	0.071^{ns}	0.019^{ns}
B x P x C	16	3.37^{ns}	8.116^{ns}	0.074^{ns}	0.032^{ns}
Residual	135	6.62	9.561	0.110	0.030
C.V. (%)		19.5	72.5	21.3	26.1

ns, not significant; *, **- significant at $p \leq 0.05$ and $p \leq 0.01$ by F-test, respectivel shoot dry weight to root dry weight ratio (SDW/RDW); while concentration showed a significant interaction with all the variables except the shoot dry weight to root dry weight ratio (SDW/RDW) (Table 4).

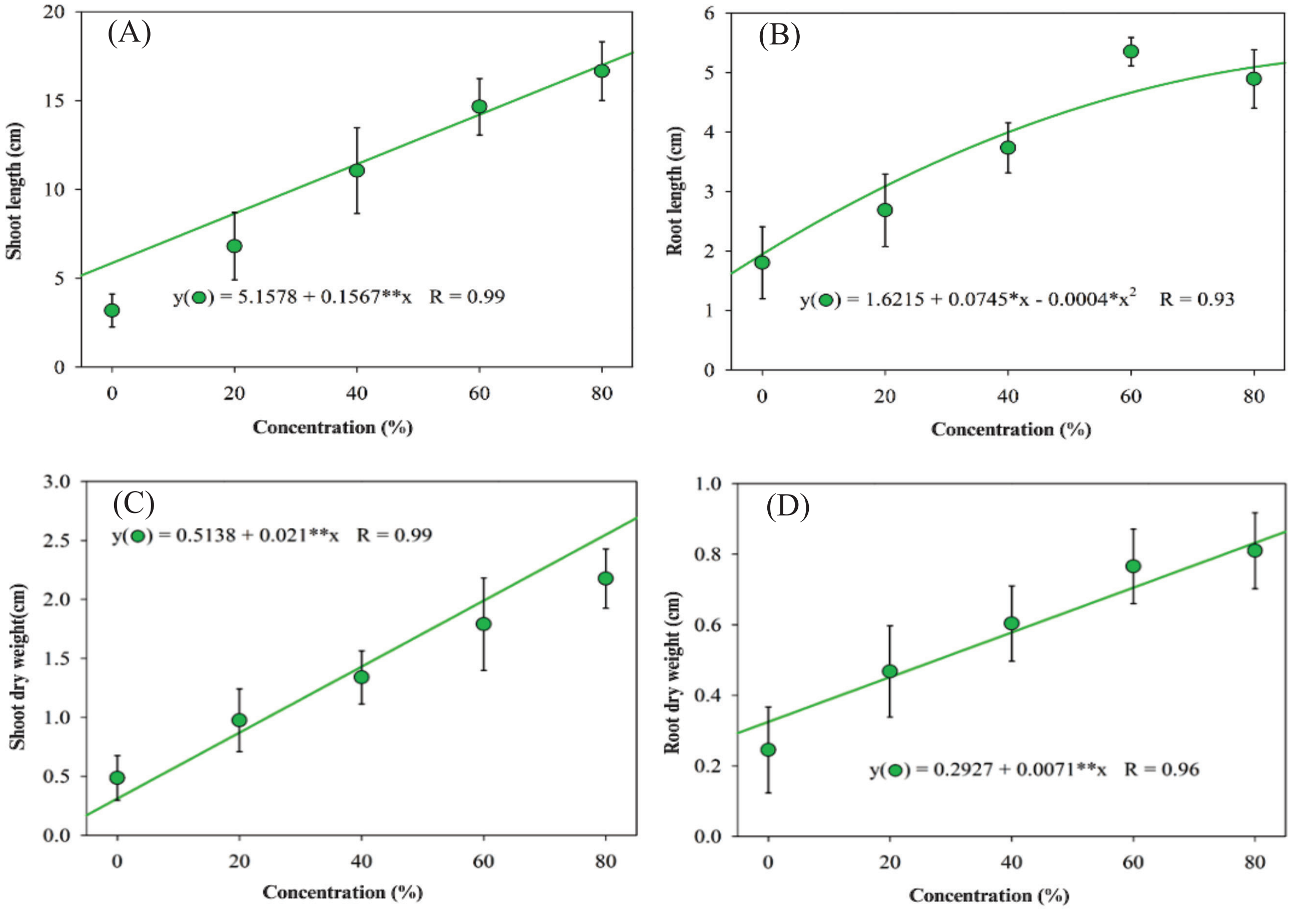
Nutrient availability in the soil has a direct effect on seedling growth. In nutrient-rich environments, plants allocate more biomass to the shoots, favouring stem development; while in poor soils, the plant invests more in the roots to maximise the uptake of water and nutrients (Mašková; Herben, 2018). For the height to diameter ratio, which expresses the plant growth balance, the shell powder concentration had a quadratic effect on the height to stem diameter ratio, with 4.05 and 9.24 for the 0% and 80% concentrations, respectively (Figure 8A). The relationship between shoot height and shoot dry weight showed a linear reduction as the concentrations increased (Figure 8B).

The height to stem diameter ratio indicates the balance of seedling development, as it associates two important variables in a single index, estimating, above all, survival in the field. Furthermore, this relationship can be used to predict the likelihood of establishment under a wide range of field conditions. Similar results were found by Paim *et al*. (2022) in *Lupinus bracteolaris* for the shoot height to shoot dry weight ratio (H/SDW).

Analysis of the correlation matrix showed strong and significant correlations (P < 0.05) between the variables (Figure 9). The positive correlation between shoot length (SL) and root length (RL), as well as between shoot dry weight (SDW) and root dry weight (RDW) are clearly evident, suggesting balanced growth.

The correlation matrix highlights the relationship between the morphological and physiological variables. There was a strong positive correlation between shoot length (SL) and root length (RL) (r = 0.89), showing that plants with greater shoot growth also tend to have greater root development. In addition, shoot dry

Figure 5 - Shoot length - SL (A) and root length - RL (B) in *Crotalaria juncea* under different concentrations of *Anomalocardia flexuosa* shell powder



weight (SDW) was highly correlated with root dry weight (RDW) (r = 0.82), suggesting that biomass accumulation is proportional. The number of leaves (NL) also showed a significant correlation with SL (r = 0.81) and RL (r = 0.72), underlining the relationship between leaf expansion and overall plant growth.

On the other hand, the emergence speed index (ESI) showed a moderate negative correlation with SDW (r = -0.47) and RDW (r = -0.43), suggesting that seedlings with a higher speed of emergence tend to accumulate less biomass during initial development. The other variables, such as the shoot height to collar diameter ratio (H/SD), shoot height to shoot dry weight ratio (H/SDW) and shoot dry weight to root dry weight ratio (SDW/RDW) showed low or insignificant correlations with the other parameters under evaluation.

Figure 6 - Variation in the length of Crotalaria juncea seedlings as a function of the different particle sizes and concentrations of Anomalocardia flexuosa shell powder, and of the base constituents used in the substrate

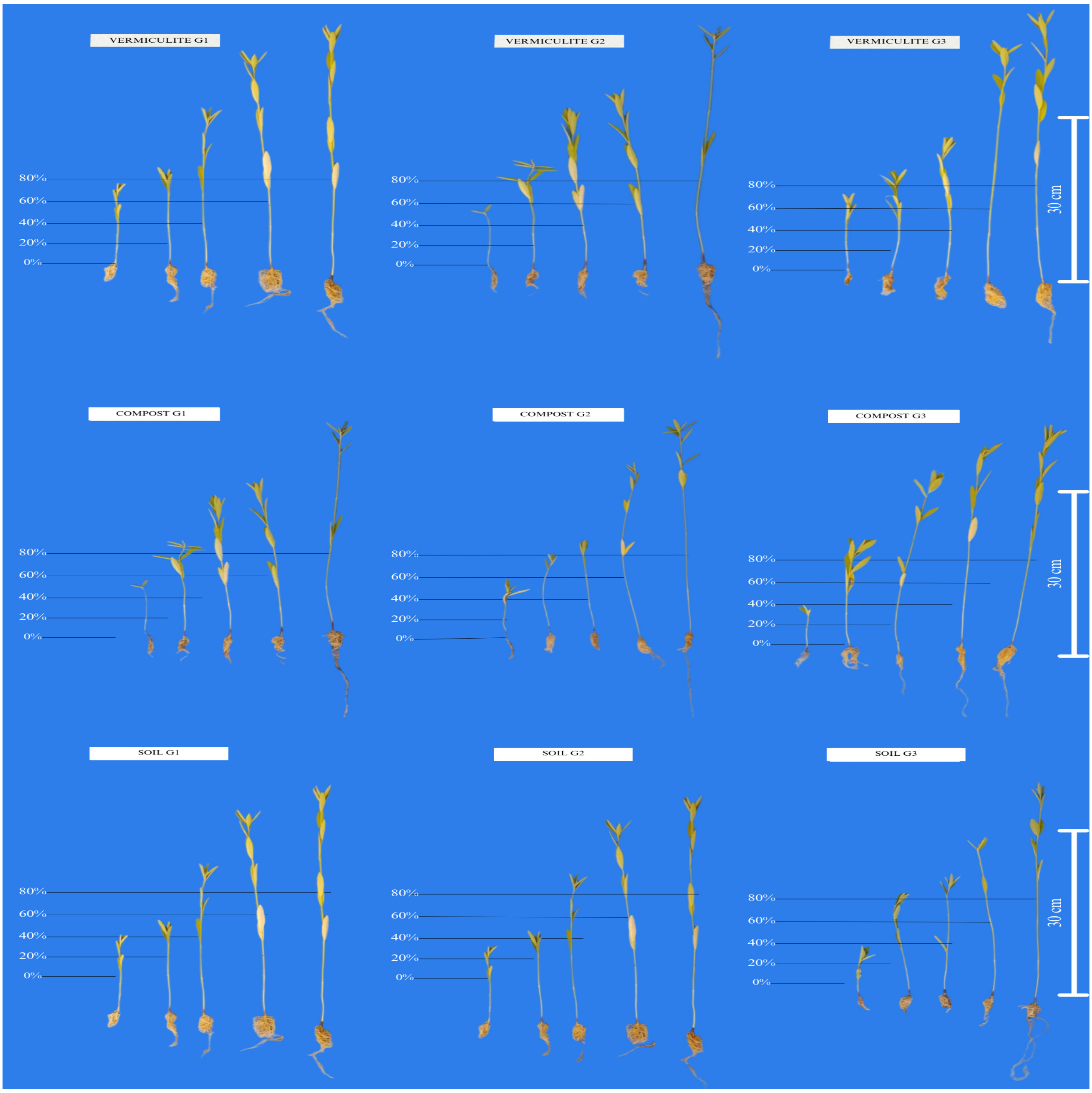


Figure 7 - Shoot dry weight - SDW (A) and root dry weight - RDW (B) in *Crotalaria juncea* under different concentrations of *Anomalocardia flexuosa* shell powder

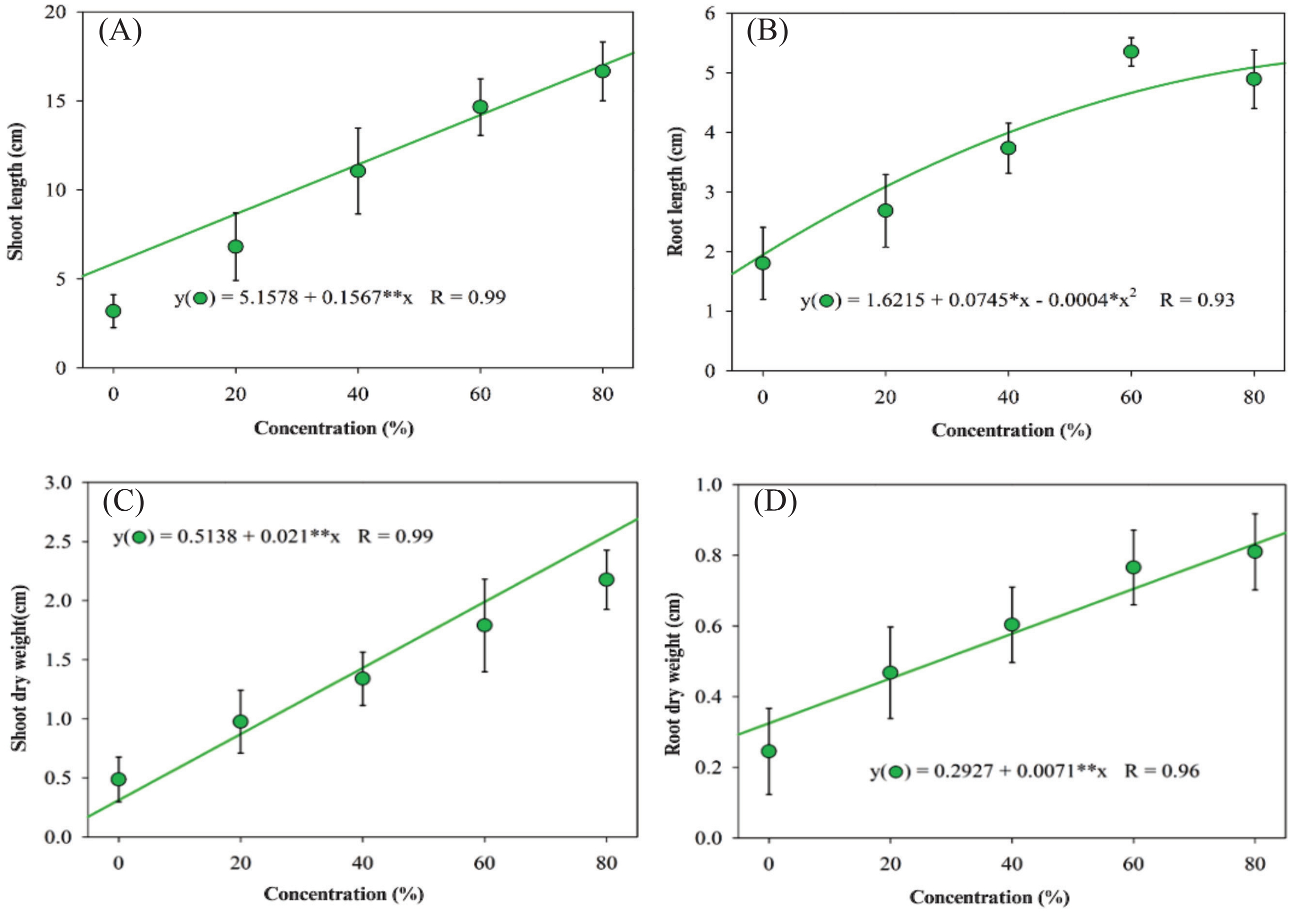


Table 4 - Analysis of variance for the shoot height to collar diameter ratio (H/CD), shoot height to shoot dry weight ratio (H/SDW) and shoot dry weight to root dry weight ratio (SDW/RDW) in seedlings of *Crotalaria juncea*

Fontes de variação	GL	Quadrado médio		
		H/DC	H/MSPA	MSPA/MSR
Granulometrias (G)	2	3.947^{ns}	28.419^{ns}	0.635^{ns}
Materiais (M)	2	6.117^{ns}	24.342^{ns}	1.143^{ns}
Concentrações (C)	4	167.239**	70.979**	1.267^{ns}
M x G	4	5.104^{ns}	14.030^{ns}	0.434^{ns}
M x C	8	2.102^{ns}	5.909^{ns}	0.820^{ns}
G x C	8	2.523^{ns}	21.340^{ns}	1.029^{ns}
M x G x C	16	2.336^{ns}	17.314^{ns}	0.889^{ns}
Resíduo	135	3.086	14.913	0.795
C.V. (%)		24.26	41.07	26.52

ns, not significant; *, **- significant at $p \leq 0.05$ and $p \leq 0.01$ by F-test, respectively

Figure 8 - Height to diameter (A) and height to shoot dry weight ratio (B) in *Crotalaria juncea* under different concentrations of *Anomalocardia flexuosa* shell powder

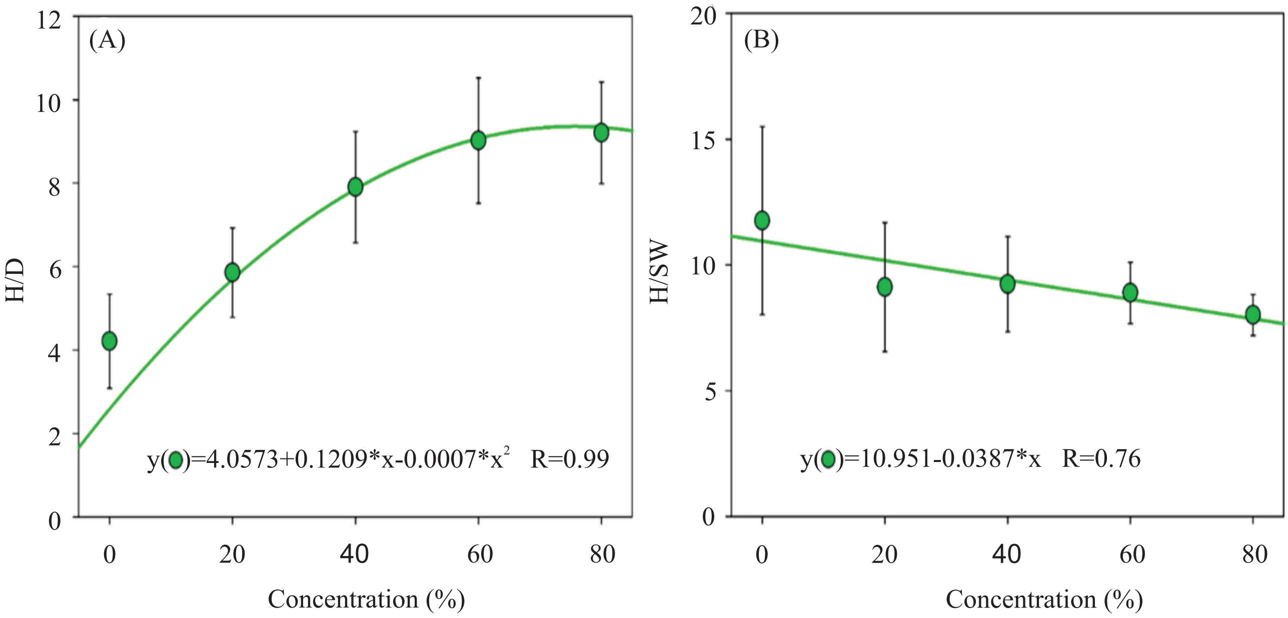
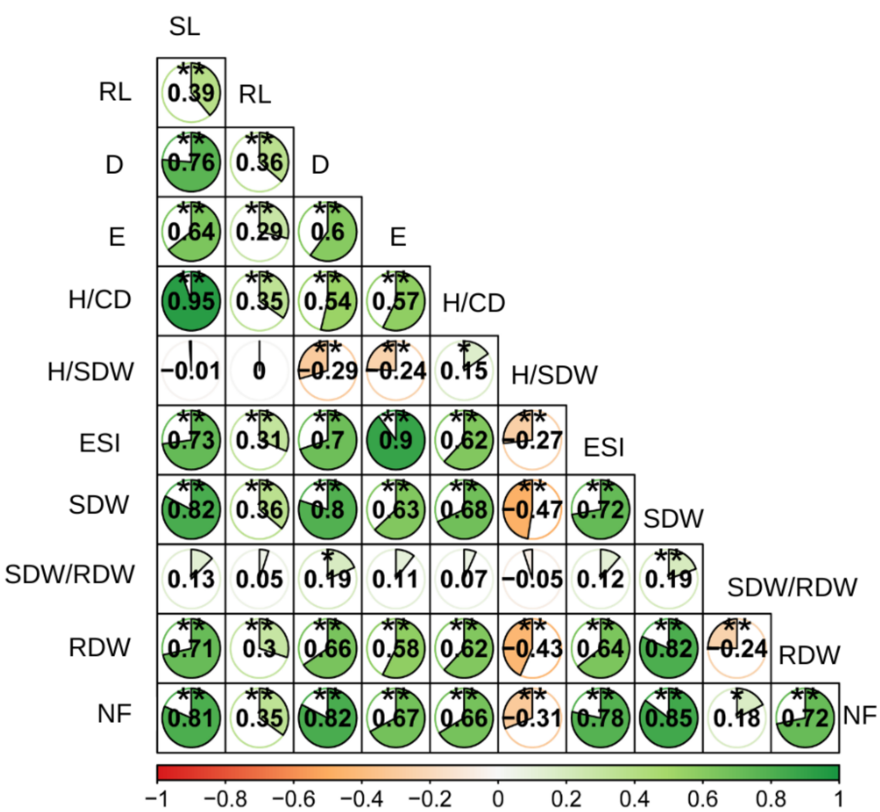


Figure 9 - Correlation matrix for the variables emergence (E), emergence speed index (ESI), shoot height to collar diameter ratio (H/CD), shoot dry weight to root dry weight ratio (SDW/RDW), shoot height to shoot dry weight ratio (H/SDW), diameter (D), number of leaves (NF), shoot length (SL), shoot dry weight (SDW), root length (RL) and root dry weight (RDW) in seedlings of *Crotalaria juncea*



CONCLUSION

An 80% concentration of shell powder in the substrate, regardless of particle size or basic constituents, enhances emergence, shoot length (SL), root length (RL), shoot dry weight (SDW) and root dry weight (RDW) in seedlings of *C. juncea*.

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